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# The expanding role of Wnt signaling in bone metabolism

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## Introduction

The quest for targetable molecules and pathways that can be manipulated to treat skeletal disease and restore bone health is perpetually evolving. Successful pharmacologic treatment of nearly all skeletal diseases among adults, and many pediatric skeletal diseases, requires a fundamental understanding of the influence that different signaling pathways exert on the bone remodeling cycle. Because pharmacologic agents that improve bone mass and fracture susceptibility can work by increasing [1] or by decreasing [2] bone turnover, it is clear that the effects of any agent on both resorptive and formation arms of the remodeling cycle are key to its success. Recently, a great deal of therapeutic interest has developed around the Wnt signaling pathway in light of the high bone mass phenotype observed among patients with certain mutations in Wnt-signaling-associated genes.

## The bone blastic bias — a straightforward path(way) to more bone

The 1997 discovery that a region on chromosome 11 was linked to very high bone mass in a single familial pedigree fueled a great deal of effort to identify the relevant gene or genes in that locus [3]. A few years later, the gene was identified as LDL-receptor related protein 5 (LRP5) — a gene (and entire pathway) that had no known role in bone metabolism at that time [4]. At almost exactly the same time, the same gene (different mutation) was identified as the culprit for very low bone mass in patients with Osteoporosis Pseudoglioma [5]. The mechanism producing both bone phenotypes appeared to be purely osteoblastic. In both cases, OPPG and HBM (and in engineered mice modeled after these conditions), osteoclast/resorption markers were normal, but bone formation was markedly altered [5,6]. Consequently, the field of Wnt in bone became focused almost exclusively on bone formation and signaling within osteoblasts, for a very good reason. First, the emerging story indicated by the clinical and experimental data was simple and powerful — low bone mass was observed in loss-of-function mutation of LRP5, and high bone mass was observed gain-of-function mutation of LRP5; almost as if LRP5 functioned as a rheostat for bone formation. Second, there was a paucity of anabolic therapies for bone, and this pathway appeared to hold great promise for targeting osteoblasts specifically. Moreover, the normally shaped bones and the absence of cancer (a long-standing concern for hyperactive Wnt signaling in other tissues) in the HBM patients was particularly attractive, though the sample sizes to support these claims were extremely low. Third, endogenous secreted inhibitors

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were known (or were subsequently found) to modulate LRP5 signaling, which makes pharmacologic targeting much more straightforward. Lastly, at least one endogenous Wnt signaling inhibitor (sclerostin) was highly selective for bone tissue—specifically osteocytes—which alleviated some of the off-target concerns of a drug targeting this protein. Further, the bone overgrowth phenotype among sclerosteosis patients appeared to make perfect sense when viewed through the lens of unrestrained LRP5 signaling, as appears to occur in the sclerostin-protected LRP5 HBM-causing mutations. All of these factors generated excitement about the Wnt pathway in osteoblast biology, and stimulated numerous research programs, both academic and commercial, to focus on Wnt signaling in osteoblasts.

## Wnts launch into osteoclast territory, without a cannon?

The excitement over Wnt signaling in osteoblasts, and its therapeutic potential, diverted attention away from the osteoclast field. The majority of osteoclast work in relation to Wnt signaling in general was centered around the observation that Wnt signaling in the osteoblast-lineage cells per se controlled osteoclastogenesis via modulation of the RANKL/OPG signal output from these cells. For example, modulation of Wnt 3A [7], sFrp1 [8], or β-catenin [9,10] among osteoblast lineage cells alters osteoclast maturation and activity in a RANKL/OPG dependent manner. But the past two years have witnessed an increased interest in direct Wnt signaling in osteoclast biology. Much of the cellular machinery is present in osteoclasts to carry out canonical Wnt signaling. Osteoclast progenitors and mature osteoclasts express LRP6 abundantly (but do not express LRP5) [10]. Intracellular signaling is also intact. In vivo, heterozygous expression of a non-degradable β-catenin mutant in osteoclasts (using PPARg-driven Cre) drastically reduces osteoclast numbers and resorption, as does Gsk3β inhibition in vitro [11]. Conversely, heterozygous deletion of endogenous β-catenin (using the same Cre driver) enhances osteoclast numbers and resorption [11]. These mutations induced drastic changes to the bone tissue, which complicate the interpretation of their effects, but it appears that β-catenin impacts the osteoclast life cycle by altering the transition from quiescent to proliferating to differentiated cells. However, earlier differentiation checkpoints might not be affected, as β-catenin does not appear to be involved in the transition from HSC to early myeloid lineage cells; β-catenin deficient HSCs transplanted into irradiated $CD45.1^{+}$ mice were capable of producing the normal number of myeloid lineage cells (and all other HCS-derived lineages) [12] Here we must draw the distinction between Wnt/β-catenin signaling and β-catenin signaling in the strict sense, as other inputs beyond Wnt/Lrp/Dsh can alter β-catenin activity (e.g. Akt, Pka, mTor).

More recently, *non-canonical* Wnt signaling in osteoclasts has been put forth as a crucial cascade in osteoclastogenesis, involving osteoblast-derived Wnt5a stimulating the non-canonical Wnt receptor Ror2 on osteoclasts [13]. The Wnt5a effect on osteoclast formation in vitro was observable only in the presence of RANKL (i.e., RANKL-dependent), and was abolished in Ror2-deficient osteoclasts. Interestingly, Wnt 5a signaling through Ror2 in osteoclasts controls the expression of RANK, which might enhance the osteoclast's responsiveness to RANKL. The most recent contribution to Wnt signaling in osteoclast biology appears in this issue of *Bone*, where Wan et al. examine the effect of impaired Wnt secretion from the osteoblast/osteocyte (Obl/Ocy) population on osteoclast differentiation

[14]. Rather than knocking down each of the 19 Wnt genes individually, these authors prevented Wnt release from bone cells by knocking out the Wntless gene (Wls) in osteoblasts and osteocytes, a strategy that had worked well in previous reports [15]. Wls controls the movement of Wnt proteins through the secretory pathway, and ultimately, release of Wnts from the cell. In vivo recombination of the floxed Wls alleles in Col1α1-expressing cells resulted in significant increases in osteoclast number, osteoclast progenitors in the marrow, and serum RANKL and CtX—suggesting that one or more unidentified Wnts secreted from osteoblasts and/or osteocytes normally suppress osteoclastogenesis and resorption. Interestingly, addition of the non-degradable β-catenin mutant allele (discussed above) in these Obl/Ocy Wntless mice reduced osteoclast number significantly, but not to the same degree as was observed in the β-catenin mutants, despite yielding no difference in RANKL or OPG expression between these models. Those data suggest that Wnts might have a role—albeit minor—in RANKL *independent* osteoclastogenesis. Probing this phenomenon further, Wan et al. found that wild-type bone marrow monocytes co-cultured with osteoblasts from the Col1α1-Cre Wls mice were better able to differentiate into osteoclasts than the same pool of monocytes co-cultured with osteoblasts from wild-type mice. Because RANKL, OPG, and M-CSF expression were not significantly different in the mutant and wild-type osteoblast support cells, the authors suggest that the observed mechanism is independent of the RANKL/OPG axis. While these experiments might benefit from additional future confirmatory work (e.g. neutralization of the RANKL signal from support cells, osteoclastogenesis in Wls and RANKL deficient mice), it is nonetheless provocative to consider that modulation of Wnt signaling might be harnessed to control both arms of the remodeling cycle, rather than its currently-held status as a modulator of osteoblast-mediated bone modeling. Further, whether the osteoclast effect observed by Wan et al. involves canonical mechanisms, one or more noncanonical mechanism(s), or a combination of both (presumably the release of both canonical and noncanonical Wnts was impaired by the Wls mutation), will require additional experiments to sort out.

As new Wnt-related therapies are developed to treat skeletal disease [16], a broader understanding of their effects on skeletal homeostasis is necessary. This is particularly pertinent as therapeutics that modulate the pathway move from canonical target receptors (e.g., sclerostin, LRP5) to canonical/noncanonical ligands. For example, targeting sFrps has therapeutic potential, but it is unclear whether the sFrps have greater affinity for certain Wnts that might participate in osteoclastogenesis versus those that are involved in modulating canonical Wnt signaling in osteoblasts and osteocytes (or both). Ironically, the experimental challenges associated with trying to understand Wnt signaling when dealing with so many different Wnt genes, might in the end prove to be an asset if the bone turnover can be finely manipulated by selectively accessing certain, or different combinations of, Wnt proteins.

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